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Accumulation of weathered polycyclic aromatic hydrocarbons (PAHs) by plant and earthworm species

Zakia D. Parrish [a], Jason C. White [a,*], Mehmet Isleyen [b], Martin P.N. Gent [c], William Iannucci-Berger [b], Brian D. Eitzer [b], Jason W. Kelsey [d], Maryjane Incorvia Mattina [b]

[a] *Department of Soil and Water, Connecticut Agricultural Experiment Station (CAES), 123 Huntington Street, New Haven, CT 06504, United States*

[b] *Department of Analytical Chemistry, CAES, 123 Huntington Street, New Haven, CT 06504, United States*

[c] *Department of Forestry and Horticulture, CAES, 123 Huntington Street, New Haven, CT 06504, United States*

[d] *Program in Environmental Science, Department of Chemistry, Muhlenberg College, Allentown, PA 18104, United States*

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Abstract

Experiments were conducted to assess the bioavailability of polyclycic aromatic hydrocarbons (PAHs) in soil from a Manufactured Gas Plant site. Three plant species were cultivated for four consecutive growing cycles (28 days each) in soil contaminated with 36.3 μg/g total PAH. During the first growth period, *Cucurbita pepo* ssp. *pepo* (zucchini) tissues contained significantly greater quantities of PAHs than did *Cucumis sativus* (cucumber) and *Cucurbita pepo* ssp. *ovifera* (squash). During the first growth cycle, zucchini plants accumulated up to 5.47 times more total PAH than did the other plants, including up to three orders of magnitude greater levels of the six ring PAHs. Over growth cycles 2–4, PAH accumulation by zucchini decreased by 85%, whereas the uptake of the contaminants by cucumber and squash remained relatively constant. Over all four growth cycles, the removal of PAHs by zucchini was still twice that of the other species. Two earthworm species accumulated significantly different amounts of PAH from the soil; *Eisenia foetida* and *Lumbricus terrestris* contained 0.204 and 0.084 μg/g total PAH, respectively, but neither species accumulated measurable quantities 5 or 6 ring PAHs. Lastly, in abiotic desorption experiments with an aqueous phase of synthetically prepared organic acid solutions, the release of 3 and 4 ring PAHs from soil was unaffected by the treatments but the desorption of 5–6 ring constituents was increased by up to two orders of magnitude. The data show that not only is the accumulation of weathered PAHs species-specific but also that the bioavailability of individual PAH constituents is highly variable.
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Keywords: Phytoremediation; Polycyclic aromatic hydrocarbons; PAHs; Uptake; *Cucurbita pepo*; Zucchini

1. Introduction

Manufactured Gas Plants (MGP) were operated as energy-generating facilities in the United States (US) up until the early 1900s, but the large amount of waste and soil contamination associated with these installations is a current issue of environmental concern. Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants that have been released anthropogenically into the environment through industrial processes such as MGP sites, wood treatment facilities, household heating, and road transport (Rost et al., 2002). PAHs are of concern because of their toxicity, persistence, and recalcitrance to remediation. The US Environmental Protection Agency regulates sixteen PAHs as priority pollutants, seven of which are known carcinogens. Certain PAHs are also classified as persistent organic pollutants (POPs) and persistent bioaccumulative

* Corresponding author.
E-mail address: Jason.White@po.state.ct.us (J.C. White).

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chemicals (PBTs), categories that also include polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins/dibenzo-*p*-furans (dioxins/furans), and several organochlorine pesticides such as DDT/DDE/DDD and chlordane (Ritter et al., 1995). PAHs vary widely in molecular structure, the most common of which range from 2 to 6 fused rings, and in other physicochemical properties such as water solubility, which ranges from 31 mg/l for naphthalene (2 rings) to 290 ng/l for benzo(*g*,*h*,*i*)perylene (6 rings) (Sims and Overcash, 1983). PAH fate in soils is also variable, ranging from biodegradable to recalcitrant. Shuttleworth et al. (1995) reported a phenanthrene half-life (3 rings) in soil and sediment of 16–126 days, but benzo[*a*]-pyrene (five rings) ranges from 229 to over 1400 days. A number of remediation options are available for lower molecular weight PAHs, but the inherent molecular recalcitrance and extreme hydrophobicity of the 5–6 ring constituents dramatically limit the success of treatment technologies (Potter et al., 1999).

Phytoremediation is an in situ technique in which the inherent physiological processes of vegetation and associated microorganisms are used to extract, degrade, or sequester organic and inorganic pollutants in/from water and soil (Cunningham et al., 1996; Schnoor, 2002). The specific mechanism by which the plant drives remediation is dependent on the type of contaminated media and the nature of the contaminant, i.e. whether it is an organic or inorganic compound. Inorganic contaminant phytoremediation mainly addresses heavy metals and is a phytoextraction from soil followed by sequestration of the pollutant within vegetative tissues (Lasat, 2002). There are numerous studies utilizing a variety of plant species in the phytoremediation of heavy metals, including Cu, Zn, Pb, Ni, Cd, Se, and As (Lasat, 2002). Plants may mediate organic contaminant biodegradation either directly through vegetative metabolic processes (phytotransformation), or indirectly by stimulation of rhizosphere bacteria that metabolize target compounds (enhanced rhizosphere degradation) (Schnoor, 2002). Hydrophilic organic compounds such as TNT (Thompson et al., 1998), atrazine (Burken and Schnoor, 1996) and carbon tetrachloride (Wang et al., 2002) can be absorbed by the plant in the transpiration stream, where the contaminants are subsequently degraded or transpired. Hydrophobic organic compounds ($\log K_{ow} > 3.5$) are bound strongly to the soil or root surfaces, making accumulation within plants unlikely (Wild and Jones, 1992; Simonich and Hites, 1995; Schnoor, 2002). The ex planta degradation of contaminants by rhizosphere microorganisms has been described for chlorinated solvents (Siciliano et al., 1998), certain herbicides (Alvey and Crowley, 1996; Boyle and Shann, 1998) and some PAHs (Aprill and Sims, 1990; Banks et al., 1999). Although the transformation of low molecular weight PAHs (3 or less rings) has been shown to be effective, the degradation of the 4–6 ring constituents is limited by low aqueous solubility and high rates of sorption to the soil (Potter et al., 1999).

Conversely, *Cucurbita pepo* ssp. *pepo* (zucchini and pumpkin) will extract weathered POPs such as chlordane, *p*,*p*′-DDE, dioxins/furans, and PCBs from soil and translocate those compounds to their above ground tissues in a process analogous to heavy metal phytoextraction (Hülster et al., 1994; Mattina et al., 2002; White et al., 2002, 2003a, in press). The mechanism of this unique POP uptake process is currently unclear but likely involves separate ex planta and in planta processes. We have previously hypothesized that this uptake ability may in part result from a unique mode of exudation of low molecular weight organic acids (LMWOA) as part of a nutrient acquisition strategy. LMWOAs have been shown to disrupt the sequestering soil matrix, thereby promoting the increased availability of weathered POPs (White and Kottler, 2002; White et al., 2003b). Preliminary data from our laboratory has shown that in soil, *C. pepo* ssp. *pepo* exudes twice the amount of LMWOAs as do other cucurbits (Eitzer et al., Submitted for publication). The mechanism by which highly hydrophobic organic contaminants are translocated from roots to shoots remains unknown. However, the primary objective of the current study is to determine whether the unique phytoextraction processes of *C. pepo* ssp. *pepo* extend to other hydrophobic organic compounds such as 5–6 ring PAHs.

A greenhouse pot study was conducted to quantitate the phytoextraction of high molecular weight PAHs from a weathered soil over sequential planting cycles with multiple plant species. For comparison, the accumulation of weathered PAHs by two worm species, *Eisenia foetida* and *Lumbricus terrestris*, in the same soil was determined. Lastly, two desorption experiments were conducted to evaluate the effects of plant-derived and synthetic organic acids on the availability of different PAH constituents in soil.

2. Materials and methods

2.1. PAH accumulation by plant species

2.1.1. Experimental design

Soil was collected from a manufactured gas plant (MGP) in Rockville, Connecticut that is contaminated with 12 of the 16 EPA Priority PAHs. Plastic pots lined with filter paper were filled with 1 kg of MGP soil. The soil has an organic carbon content of 3.5% (loss on ignition) and a pH of 6.0–6.1 (Yang et al., 2001). There were four treatments: an unvegetated control and three different plant species that have demonstrated different degrees of uptake and translocation ability in *p*,*p*′-DDE contaminated soil (White et al., 2003a). Each treatment had three replicates in a complete randomized block design in a greenhouse (12 pots total).

Seeds of two cultivar varieties of *C. pepo*; a zucchini (cv. Black Beauty) and a summer squash (cv. Zephyr), and a cucumber (*Cucumis sativus* cv. Marketmore), purchased from Johnny's Selected Seeds (Albion, ME), were placed in a clear plastic box on top of moistened germination

paper and kept at room temperature for five days. Four seedlings of each species were transferred to their respective pots and allowed to grow for one week, after which each pot was thinned to one plant.

There were two sampling periods during each of the four cycles, at zero and four weeks. At the initial sampling period, 10 g of soil was collected from each pot in 40-ml amber glass vials with Teflon-lined screw caps for PAH analysis. At the final sampling period, the entire plant was sacrificed for PAH analysis. The aboveground biomass was removed and the leaves and stems were analyzed separately. The roots were separated from the soil and analyzed with the other vegetation. The vegetation was washed with water to remove any soil, and the wet mass of all tissue compartments was determined. The replicates were composited according to treatment and tissue type, finely chopped, and stored at −4 °C in 250-ml amber glass jars with Teflon-lined screw caps until extracted.

After the vegetation was harvested, the soil from each of the replicates of a single plant cultivar were combined and thoroughly mixed in a large bin by hand and with the use of a garden trowel. The pots were then refilled with approximately one kilogram of that soil. This process was carried out for each of the treatments in preparation for the start of the next growth cycle. The pots were replanted with seedlings from the same plant species as in the previous cycle and allowed to grow for an additional four weeks prior to harvest. This procedure was repeated three times, resulting in four growth cycles.

During cycle two, the plants began to show signs of nutrient deficiency; so 30-ml of 10% Hoagland's solution was applied to each of the pots weekly during the last three weeks of both cycles three and four. During cycle four, rhizosphere soil was collected from the roots. The recovered roots were gently shaken to remove any excess soil, then allowed to air-dry, after which any additional soil that was removed was considered 'rhizosphere' soil and was collected in a separate 20-ml amber glass vial with a Teflon-lined screw cap.

2.1.2. PAH extraction from soil

Soil samples were extracted by methods similar to those described in White et al. (2003a). The soils were air-dried for 24 h, and 3-g of soil from each treatment were weighed into 40-ml amber glass vials. Fifteen milliliters of hexane and 100-μl of a 10-μg/ml internal standard solution were added to the vials, which were then sealed with Teflon-lined screw caps and placed in a 70 °C oven for 5 h. For cycles 1 and 2, $^{13}C_6$-phenanthrene was used as an internal standard; however, it was replaced in cycles 3 and 4 with d_{10}-phenanthrene due to concentration limitations and cost. The vials were removed from the oven, allowed to cool for 10 min and a 1-ml aliquot was transferred to a GC vial through a 0.2 μm glass microfiber filter. An additional 3-g of each soil was weighed into individual aluminum tins and placed in a 105 °C oven for 24 h for moisture content determination.

2.1.3. Vegetation extraction

PAHs were recovered from the plant tissues using the extraction method developed by Pylypiw et al. (1993). Approximately 0.5–2 g of vegetation (wet weight), 10-ml of 2-propanol, and 100-μl of either 10-μg/ml $^{13}C_6$-phenanthrene (cycles 1 and 2) or 50-μg/ml d_{10}-phenanthrene (cycles 3 and 4), as internal standard, was added to one-quart blender jars and blended for 30 s at high speed. Twenty milliliters of petroleum ether was added, and then blended for 5 min at high speed. The contents were allowed to settle for 2–3 min, after which the extract was passed through a glass wool packed glass funnel into a 500-ml glass separatory funnel with a Teflon stopcock. The extract was allowed to drain for 15 min, and then 30-ml of reverse osmosis water (RO-water) and 3-ml of saturated sodium sulfate solution were added to each funnel. The funnels were capped, shaken for 10 s, and the phases allowed to separate for 15 min. The water layer was drawn off and the remaining petroleum ether was rinsed twice with 30-ml of RO-water alone, and one final time with 10-ml RO-water and 1-ml of saturated sodium sulfate solution, drawing off the water layer after each rinse. The petroleum ether was transferred to a graduated cylinder containing 3 g of anhydrous sodium sulfate and allowed to sit for 1 h to remove any additional water in the extract. Two, 1-ml aliquots were transferred to GC vials through 0.2 μm glass microfiber filters and stored at 4 °C until analyzed via GC–MS. Approximately 0.5–1 g of vegetation was weighed into aluminum tins and placed in a 100 °C oven for 24 h for moisture content determination.

2.2. Uptake of PAHs by earthworms

The uptake of weathered PAHs by two species of earthworms was determined: *E. foetida* and *L. terrestris* (obtained from Carolina Biological Supply, Burlington, NC). Five hundred grams of contaminated soil was added to 600-ml beakers. Approximately 3 g (wet weight) of biomass was added to replicate soil samples, 30 individuals of *E. foetida* or three individuals of *L. terrestris*. Each species was tested in triplicate beakers of soil. The earthworms were washed with tap water prior to their addition to the tops of the soil samples. Beakers were covered with aluminum foil that was secured with rubber bands, and four small holes were cut in the foil for aeration. The soil samples were then stored in the dark at 22 °C. After 14 days, the worms were removed from the soil, washed with tap water, and transferred to clean petri dishes for depuration (24 h for *E. foetida* and 48 h for *L. terrestris*). At harvest, the worms were divided into nearly equal portions (5–7) by mass and transferred to 35-ml vials containing 10 ml of hexanes and 100-μl of 10-μg/ml d_{10}-phenanthrene. The vials were sealed with Teflon-lined closures and were placed upright in an oven at 70 °C for 5 h. The samples were removed from the oven, and a 1-ml aliquot of the supernatant was passed through a glass microfiber filter (0.2 μm,

Laboratory Science Inc., Sparks, NV) for particulate removal prior to analysis.

2.3. Abiotic desorption of PAHs

2.3.1. Synthetic organic acid solutions

One-liter solutions were prepared in deionized distilled (DDI) water containing malic, citric, and succinic acid at concentrations ranging from 0 to 15 000 mg/l. For each solution, the initial pH was measured and ranged from 2.63 to 5.53. To assess the influence of pH, half of the solutions then buffered using NaOH until the final pH was between 6.5 and 7. A control, which contained DDI water only was prepared and the pH was not adjusted.

Ten-gram portions of soil were weighed into 70-ml vials, with each treatment having five replicates, and 50-μl of 10 μg/ml $^{13}C_6$-phenanthrene was added to each vial as an internal standard. Sixty milliliters of the solutions containing either organic acids or DDI water were added to the vials, which were sealed and put on a rotary shaker at 20 rpm for three days. The samples were removed from the shaker and centrifuged at 600 rpm for 12 min, after which the supernatant was collected and allowed to settle overnight.

A C-18 (octadecyl) disk (3M, St. Paul, MN) was added to each of the supernatants, which were then put on the shaker for three days. The C-18 disks were removed and transferred to 8-ml vials containing sodium sulfate. Four milliliters of hexanes were added to the vials, which were put in a 70 °C oven for 2 h. One hundred microliters of each sample was transferred to a GC micro-vial and analyzed via GC–MS.

2.3.2. Hydroponically isolated root exudates

Black Beauty, Marketmore, and Zephyr seeds were placed in clear plastic boxes on top of moist germination paper for five days. The seedlings were transferred to troughs containing a complete nutrient solution. Six one liter stock solutions containing 27.2 g KH_2PO_4, 101.1 g KNO_3, 189 g $Ca(NO_3)_2 \cdot 4H_2O$, 48.26 g $MgSO_4$, 2 g Fe-EDTA, and trace nutrients (1.24 g H_3BO_3, 0.676 g $MnSO_4 \cdot H_2O$, 0.115 g $ZnSO_4 \cdot 7H_2O$, 0.100 g $CuSO_4 \cdot 5H_2O$, 0.024 g MoO_3) were prepared separately. The complete nutrient solution containing a 2.5-ml aliquot of each stock solution diluted to 1-l with DDI water. Once the plants had three true leaves, they were transferred to a flowing solution in which KH_2PO_4 was replaced with KCl until the plants stopped growing due to phosphorus depletion. The plants were transferred to a 250-ml amber Qorpak jar and held static with foam plugs. Four seedlings of each cultivar were in jars containing the KH_2PO_4 nutrient solution and four were in jars containing the KCl nutrient solution. Air was supplied to the plant roots through plastic tubing fitted with a glass micropipette inserted into each jar. The plants were kept in a growth room at 25 °C with a 12-h photoperiod and the solutions were replenished as needed.

The roots were immersed in 250 ml of an antibiotic solution containing 50 mg/l streptomycin and 25 mg/l chlorophenicol in 0.25 mM $CaCl_2$ for 1 h, then transferred to a second 250-ml jar of 0.25 mM $CaCl_2$ for another hour. The roots were transferred to 250 ml of fresh 0.25 mM $CaCl_2$ for 24 h, after which the solution was collected from each cultivar for the desorption experiment.

Ten grams of soil was weighed into 60-ml vials, with each treatment having five replicates, and 100-μl of 10-μg/ml d_{10}-phenanthrene was added to each vial as an internal standard. Sixty milliliters of either the organic acid solutions collected from each plant species, 0.25 mM-$CaCl_2$, KH_2PO_4 nutrient solution, or KCl nutrient solution were added to the vials, which were sealed and put on a rotary shaker for two days. The samples were removed from the shaker and centrifuged at 1000 rpm for 12 min, after which the supernatant was collected.

A C-18 (octadecyl) disk was added to each of the samples and the solutions were extracted as described above.

2.4. PAH quantitation

The vegetation and soil extracts were analyzed for 12 of the 16 EPA Priority PAHs using an Agilent (Avondale, PA, USA) 6890 gas chromatograph (GC) with an Agilent 5973 mass selective detector (MSD). The column (30 m × 250 μm × 0.25 μm) contained a MDN-12 film (Supelco, Bellefonte, PA) and the GC program was 80 °C initial temperature, held for 1 min, then ramped at 15 °C/min to 350 °C and held for 10 min. The total run time was 27 min. A 2-μl splitless injection was used, the injection port was maintained at 300 °C and the MS detector was maintained at 280 °C.

Crystalline d_{10}-phenanthrene and 100 μg/ml $^{13}C_6$-phenanthrene in *n*-nonane were acquired from Cambridge Isotope Laboratories (Andover, MA). A stock of the 16 EPA Priority PAHs at 2000 μg/ml in methylene chloride/benzene (1:1) was acquired from Ultra Scientific (North Kingstown, RI). The PAH stock solution was diluted with hexane to prepare calibration standards for cycles 1 and 2 at 25, 50, 100, 250, 500, 1000, 2500, 5000, 7500, 10 000 ng/ml and each calibration level was amended with 5-μg/ml $^{13}C_6$-phenanthrene as an internal standard. For cycles 3 and 4, as well as the abiotic desorption and earthworm experiments, the PAH calibration standards were prepared at 50, 75, 100, 250, 500, 1000, 2500, and 5000 ng/ml and each calibration level was amended with 100 ng/ml d_{10}-phenanthrene as an internal standard. Both soil and vegetation PAH concentrations are reported on a dry weight basis.

2.5. Statistical analysis

All concentrations of PAHs in biomass and soil are reported on a dry weight basis. Differences in contaminant content or biomass were determined by a one way analysis of variance on ranks followed by a Student Newman Keuls

multiple comparison test with $p < 0.05$ (SigmaStat, SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Soil analysis

Replicate soil extractions confirmed the presence 12 weathered 3–6 ring PAHs (Table 1) for a total concentration of 36.3 μg/g; other PAHs were analyzed for but the listed contaminants were the only constituents present at quantifiable levels. The reason for the relatively large degree of variability in the replicate extractions is unknown but is likely due to non-heterogeneous contamination of the soil on a micro- or nano-scale that is characteristic of a historically contaminated site. Thus, in spite of this moderate heterogeneity in PAH distribution and the likely associated difficulties with statistical analysis, the soil does represent the realistic contamination scenario encountered in the field. The PAH content of the soil associated with each species was measured after each growth cycle and these values, as well as the values from non-vegetated control pots, did not differ significantly from the initial PAH concentration in the soil. In addition, after cycle 4 the PAH content of the rhizospheres of the three species was measured and did not differ significantly from 36.3 μg/g; the values were 33.3 (±7.40) μg/g for zucchini, 26.2 (±11.6) μg/g for cucumber, and 38.9 (±28.2) μg/g for squash.

3.2. Vegetation analysis

Detectable quantities of PAHs were measured in all tissues of all plants over the four growth cycles and almost without exception, the root PAH concentration was greatest, followed by decreasing levels in the stems and leaves. The total PAH content in the tissues of the three plant species over the four growth cycles are shown in Table 2.

Table 1
PAH content of soil collected from a CT manufactured gas plant site

PAH	Number of rings	Concentration (ng/g)
Phenanthrene	3	3920 (1160)
Anthracene	3	1490 (747)
Fluoranthene	3	6520 (1990)
Pyrene	4	6260 (1890)
Benzo[*a*]anthracene	4	2480 (734)
Chrysene	4	3480 (974)
Benzo[*b*]fluoranthene	5	3490 (1050)
Benzo[*k*]fluoranthene	5	1490 (462)
Benzo[*a*]pyrene	5	2820 (835)
Dibenz[*a*,*h*]anthracene	5	578 (172)
Indeno[1,2,3-*cd*]pyrene	6	1690 (520)
Benzo[*g*,*h*,*i*]perylene	6	2060 (642)
Total		36300 (16600)

Each value is the average of 12 replicate extractions (standard deviations are in parentheses).

Table 2
Total μg of PAHs in the tissues of zucchini, cucumber, and squash over four consecutive growth cycles

Growth cycle	Root PAH content (μg)	Stem PAH content (μg)	Leaf PAH content (μg)
Cycle 1			
Zucchini	5.06A[a]	2.13A	1.13A
Cucumber	1.93B	0.009B	0.124B
Squash	0.409C	0.484C	0.631C
Cycle 2			
Zucchini	1.49A	0.733A	0.255A
Cucumber	1.39A	0.505A	0.471B
Squash	0.775A	1.41B	0.425AB
Cycle 3			
Zucchini	0.876A	0.118A	0.242A
Cucumber	0.458B	0.002B	0.247A
Squash	0.086B	0.025AB	0.172A
Cycle 4			
Zucchini	0.356A	0.317A	0.627A
Cucumber	0.032B	0.235B	0.922B
Squash	0.207C	0.790C	1.78C

[a] Within a tissue and growth cycle, values followed by different letters are significantly different (One way ANOVA on ranks followed by a Student Newman Keuls Multiple comparison test, $p < 0.05$).

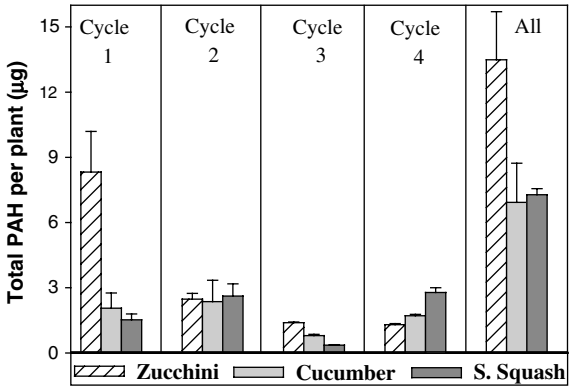

Fig. 1. Uptake and translocation of weathered PAHs by three cucurbits over multiple growing seasons.

Fig. 1 shows the whole plant PAH content for growth cycles 1–4, as well as the summed uptake over all four experiments. In the first growth cycle, the tissues of zucchini contained significantly greater levels of PAHs than did those of the other two species, including a stem content that was two orders of magnitude greater than that of cucumber. Total PAH accumulation in the zucchini was 4.04 and 5.47 times that of cucumber and squash, respectively. In terms of PAH concentration in the plants during the first growth cycle, the roots of zucchini contained 44.5 μg/g, a value significantly greater than that of the cucumber and squash, which accumulated 8.02, and 2.76 μg/g, respectively. Of particular interest was the significantly greater accumulation of 5 and 6 ring PAHs in the zucchini roots where levels were 3.00 and 4.10 μg/g, respectively. In the cucumber and squash roots, the concentration

of 5 ring PAHs was 0.970 and 0.520 μg/g, respectively, and of 6 ring PAHs was 0.640 and 0.004 μg/g, respectively.

Interestingly, in growth cycles 2–4, significant differences in PAH accumulation among the plant species were far less evident. When comparing the different species during growth cycles 3 and 4, PAH accumulation in the roots of zucchini was significantly greater than that of the other two species; there were no consistent patterns of significance with regard to the uptake of the higher molecular weight constituents. In addition, there were no consistent patterns of statistical significance between the separate species during the last three growth periods in either the contaminant concentration in the aerial tissues or in the total amount of PAH in the plants. However, in summing the PAH removal over the four separate uptake periods, zucchini did accumulate twice the amount of contaminant as did the cucumber and squash. Table 3 shows the statistical analysis on the amount of PAH removed by each of the plants over the four cycles and it is evident that zucchini exhibits a pattern clearly different from the other two species. With the exception of cycle 3, the cucumber and squash either increase or remain constant in the accumulation of PAHs whereas zucchini's ability to extract the contaminants declines markedly after the first planting. Over the four growth cycles, total PAH removal by zucchini approximated 0.07% of the soil burden. This value clearly does not approach the level required to reach typical remediation goals in a timely fashion but it is noteworthy that Wang et al. (2004) showed that the zucchini phytoextraction system for weathered organochlorine insecticides such as DDE is maximized under field conditions, not small pot cultivation.

The literature is replete with studies on the phytoremediation of PAHs, but these reports consistently focus on contaminant degradation in the plant rhizosphere and frequently, the impact on the fate of higher molecular weight 5 and 6 ring PAHs is negligible. Aprill and Sims (1990) reported slightly increased degradation of four and some five ring PAHs in the root zone of prairie grasses as compared to unvegetated soils. Similarly, Pradhan et al. (1998) noted that alfalfa and two grasses enhanced the degradation of total PAHs in one soil by up to 57%, but the degradation of "carcinogenic" high molecular weight constituents was much more modest. In addition, in a second soil, vegetation had no effect on contaminant fate.

Table 3
Total PAH accumulation (μg) in zucchini, cucumber, and squash over four growing periods

Growth cycle	Zucchini	Cucumber	Squash
Cycle 1	8.33A[a]	2.06A	1.52A
Cycle 2	2.48B	2.36A	2.62B
Cycle 3	1.24B	0.707B	0.254C
Cycle 4	1.30B	1.71A	2.78B

[a] Within a plant species, values followed by different letters are significantly different (One way ANOVA on ranks followed by a Student Newman Keuls Multiple comparison test, $p < 0.05$).

Banks et al. (1999) noted that in soils freshly amended with benzo(*a*)pyrene, 53% of the spike remained in unvegetated soils whereas only 44% was left when tall fescue was present. Liste and Alexander (1999, 2000) noted that as many as nine separate plant species enhanced the degradation of phenanthrene or pyrene that was freshly added to soil. In soil contaminated with aged petroleum sludge, Hutchinson et al. (2001) described 35% degradation in soil columns containing tall fescue as compared 18% degradation in non-vegetated soils, but the authors did not discuss potential differences in the loss of lower and higher molecular weight constituents. Parrish et al. (2004) noted that in a MGP soil initially containing 285 mg/kg total PAH, tall fescue, annual ryegrass, and yellow sweet clover reduced contaminant concentrations by 23.9%, 15.3%, and 9.10%, respectively, over a one-year period and unlike the previous studies, a significant fraction of that loss was in 4–6 ring constituents.

Studies attempting to quantify the soil to plant transfer of weathered PAHs are far less common. Gao and Zhu (2004) reported root concentration factors for phenanthrene by 12 plant species that ranged from 0.05 to 0.67 and from 0.23 to 4.4 for pyrene; stem concentration factors for phenanthrene and pyrene ranged from 0.06 to 0.12 and 0.00 to 0.12, respectively. Although the higher values of accumulation approximate those achieved by *C. pepo* ssp. *pepo*, the PAHs in the Gao study were freshly added to the soil at concentrations that exceeded those used in the current investigation by more than an order of magnitude. Samsoe-Petersen et al. (2002) did grow vegetation in soil contaminated with weathered benzo(*a*)pyrene but the authors only analyzed the fruit tissue, where the highest concentration factors were well below 0.01.

The findings that *C. pepo* ssp. *pepo* has a significantly greater ability to accumulate hydrophobic organic pollutants such as 5–6 ring PAHs agrees with previous work from our laboratory and others. Hülster et al. (1994) initially reported that *C. pepo* ssp. *pepo* accumulated weathered dioxins in the aerial tissue through a unique soil-to-plant mechanism whereas the lower levels of contaminants in the shoot systems of other plant species was the result of aerial deposition onto the leaves. Mattina et al. (2000, 2004) reported a similar enhanced accumulation of weathered chlordane by *C. pepo* ssp. *pepo*. White (2001) and White et al. (2003a, 2005) described similar phytoextraction of weathered DDE from soil by the cucurbita subspecies; findings confirmed by Lunney et al. (2004) who noted significant DDT accumulation by *C. pepo*. Lastly, White et al. (in press) observed that *C. pepo* ssp. *pepo* accumulates and translocates greater quantities of weathered PCBs than do other plant species. Elucidating the mechanisms by which this particular plant species extracts these highly weathered hydrophobic residues from soil and subsequently translocates significant quantities from the roots to shoots remains a fundamental goal of our laboratory. We have hypothesized that the removal of the residues from the soil and accumulation in the roots of *C. pepo*

ssp. *pepo* is at least in part related to the exudation of low molecular weight organic acids as a nutrient acquisition strategy. It is known that these acids can chelate inorganic soil cations, effectively disarticulating the soil matrix to facilitate the uptake of P and other nutrients but also inadvertently increase the availability of previously sequestered POPs such as *p*,*p*′-DDE and PAHs (Yang et al., 2001; White and Kottler, 2002; Subramaniam et al., 2004). Gent et al. (2005) observed that under hydroponic conditions of P depletion, *C. pepo* ssp. *pepo* not only exuded greater quantities of citric acid than did ssp. *ovifera* but also the collected exudates from ssp. *pepo* extracted larger amounts of inorganic elements from soil when slurried. Lastly, preliminary data from our laboratory involving the in situ or direct collection of root exudates from several cucurbits growing in soil shows that the levels of LMWOA are higher for *C. pepo* ssp. *pepo* than for other cucurbits (Eitzer et al., Submitted for publication). The mechanism by which this *Cucurbita* subspecies translocates highly hydrophobic compounds from roots to shoots remains a topic of ongoing investigation. The observation that the enhanced potential of ssp. *pepo* to extract PAHs from the soil may dissipate upon consecutive growing cycles is intriguing and indicates that contaminant bioavailability in soil is still the rate-limiting step for effective phytoremediation. Current investigations are focusing on further enhancing the bioavailability of weathered POPs in soil to *C. pepo* ssp. *pepo*.

3.3. Earthworm analysis

The total PAH content of *E. foetida* and *L. terrestris* was 0.204 and 0.084 μg/g, respectively (values significantly different by student *t*-test at $p < 0.05$). Interestingly, neither worm species contained detectable levels of the 3, 5, or 6 ring PAHs; only fluoanthene, pyrene, benzo[*a*]anthracene, and chrysene were accumulated to a measurable extent. The relative amounts of each of these PAHs in the worms correlated with the amounts of each contaminant present in the soil. Fluoranthene was present at the greatest concentration in the soil and in the worms tissues, and benzo[*a*]anthracene was present at the lowest amounts in both matrices.

The literature contains numerous studies on the uptake of PAHs by worm species but methodology differences (worms species and age, incubation time, worm-to-soil ratio, contaminant concentration and age) and variable data expression (concentrations that may or may not be normalized to lipid content, ratios of lipid normalized to organic carbon normalized content) make direct comparisons problematic. Johnson et al. (2002) reported a concentration ratio of pyrene and benzo(*a*)pyrene of near unity in *Aporrectodea longa* but the contaminants were freshly added to the soil, creating the confounding factors of dermal absorption and increased PAH availability when comparing to the current study. Krauss et al. (2000) reported biota-to-soil accumulation factors (BSAFs) of 0.13–0.41 for *L. terrestris* in soils containing weathered PAHs but BSAFs express the lipid normalized PAH content to the organic carbon normalized contaminant content in the soil. Using a lipid content of 1.2% of *L. terrestris* (Krauss et al., 2000) and 1.8% for *E. foetida* (Wagman et al., 2001), BSAFs can be calculated in the current study; the value for *E. foetida* is 0.011 and *L. terrestris* is 0.007 (significantly different by student *t*-test, $p < 0.01$). These findings are more in line with Matscheko et al. (2002), who described a BSAF of 0.02 for *E. foetida* but this value is based on soil organic matter content, instead of carbon. Alternatively, Jager et al. (2005) observed BSAFs of 0.23 for *E. andrei* incubated in a range of soils containing weathered PAHs. Interestingly, the uptake of PAHs by worm species seems to be significantly lower than that of other persistent organic pollutants. For example, Krauss et al. (2000) reported *L. terrestris* BSAFs that were 10–100 times higher for PCBs (values of 0.71–70) than for PAHs. Similarly, in a soil contaminated with weathered *p*,*p*′-DDE, Kelsey et al. (2005) reported accumulation factors for *E. foetida* and *L. terrestris* of 8.3 and 0.94, respectively. It is noteworthy that, in spite of the orders of magnitude difference in PAH and *p*,*p*′-DDE accumulation, the species difference is constant, with *E. foetida* consistently accumulating greater levels of contaminant than *L. terrestris*. The dramatic difference in PAH accumulation by plant roots and worms are also noteworthy. The highest PAH content in the worm tissues is more than an order of magnitude less than the lowest concentration in the roots of any of the plants. This difference, taken with the ability of plants to accumulate the five and six ring PAHs and the variability in worm assays described above, highlight the difficulty in obtaining relevant and accurate exposure and risk estimates in contaminated soil.

3.4. Abiotic PAH desorption

The percentage of 3–6 ring PAHs desorbed from soil by the various synthetically prepared organic acid solutions is shown in Table 4. A clear effect of pH is evident. In the non-adjusted solutions (pH was 2.63–3.86), the 150 mg/l and 1500 mg/l (with citric acid as the dominant constituent) solution consistently induced the greatest level of PAH desorption, and in all solutions, the PAH ring number was indirectly related to the amount of contaminant released. The highest organic acid content consistently induced the least amount of PAH desorption, and overall, pH and desorption were loosely correlated ($r^2 = 0.75$). In the pH-adjusted solutions (pH 6.60–7.00), the amount of contaminant desorbed was also indirectly related to PAH ring number. For the 3 and 4 ring PAH constituents, the effect of the organic acid solution on PAH desorption was largely non-significant, with the two lowest acid treatments actually depressing the desorption of the 4 ring contaminants. This pattern matched the effect on total PAH desorption; effects were non-significant with the exception of decreased desorption at the 150 mg/l and at 1500 mg/l (three acids at roughly equal mole fractions)

Table 4
Effect of synthetically prepared organic acid solutions on the desorption of 3, 4, 5, and 6 ring PAHs

Total organic acid content (mg/l)	Mole fraction (mM of malic/citric/succinic)	Percent PAH desorbed			
		3 Rings	4 Rings	5 Rings	6 Rings
Non-pH adjusted					
0	0	8.44A	5.75A	2.64A	0.01A
150	1.01/0.039/0.064	20.8B	9.96B	3.02A	0.02A
1500	2.80/3.91/3.18	7.32A	4.91A	2.09A	0.00A
1500	0.56/7.03/0.636	13.8B	10.6B	4.26A	0.00A
15000	37.5/26.1/42.4	0.26C	0.33C	0.11B	0.00A
15000	101/3.90/6.36	1.48D	0.87C	0.51A	0.57A
15000	5.60/70.3/6.36	0.26C	0.36C	0.05C	0.00A
pH adjusted					
0	0	10.1A[a]	6.40A	2.63A	0.00A
150	1.01/0.039/0.064	6.90A	4.93B	2.03A	0.00A
1500	2.80/3.91/3.18	6.38A	5.07B	3.31A	0.02A
1500	0.56/7.03/0.636	9.52A	7.41A	5.40B	0.48B
15000	37.5/26.1/42.4	7.46A	6.68A	6.95B	1.50C
15000	101/3.90/6.36	8.26A	7.51A	7.12B	2.22D
15000	5.60/70.3/6.36	7.77A	7.54A	8.36B	1.90D

[a] Within a column, values followed by different letters are significantly different (One way ANOVA on ranks followed by a Student Newman Keuls Multiple comparison test, $p < 0.05$).

level. However, some interesting patterns become evident when analyzing the 5 and 6 ring compounds. For both sets of compounds, there was a significant difference between the two separate 1500 mg/l organic acid treatments; the solution with the greater mole fraction of citric acid resulted in significantly greater contaminant desorption. For the 5 ring PAHs, there was no difference between the three 15000 mg/l treatments and the 1500 mg/l treatment; all increased desorption. However, for the 6 ring constituents, further significant differences were observed at the higher concentrations with solutions containing predominantly malic or citric acid being more effective than a solution containing roughly equal amounts of the three acids.

The results for the abiotic desorption experiments utilizing hydroponically isolated root exudates were far less dramatic. Similar to the synthetically prepared acid solutions, the number of rings in the PAH molecule was indirectly related to the amount of contaminant desorbed. For the exudates isolated from the three plant species, the absence of P in the nutrient solution did not significantly impact the desorption of weathered PAHs, regardless of ring number. In addition, the isolated exudates did not significantly increase the desorption of PAHs relatively to any of the three control solutions; $CaCl_2$ or the solutions with or without P.

As stated in Section 3.2, *C. pepo* ssp. *pepo* likely derives some of its unique contaminant phytoextraction abilities from LMWOA exudation as a nutrient acquisition mechanism. Thus, abiotic desorption experiments utilizing synthetic organic acids and isolated exudates can be useful in elucidating the ex planta processes responsible of contaminant uptake by *C. pepo* ssp. *pepo*. The findings of a significant pH-dependence on contaminant desorption agrees with the results of Yang et al. (2001), where PAH release from a MGP soil was found to be positively correlated with solution pH over the range of 2.9–7.5. The authors state that in addition to the pH effect on the release of H^+ from the acid carboxyl groups that will determine the magnitude of potential chelation, a lower pH can result in a condensed organic phase with humic and fulvic acids that are more tightly bound to the inorganic phase, subsequently limiting PAH diffusion (Yang et al., 2001). At a pH of 6–7, the effects on soil structure are reversed and the chelating potential of the organic acids is maximized. Subramaniam et al. (2004) observed that a range of organic acids significantly increased the extraction of polyvalent ions from soil, resulting in the dissolution of humic substances and an increased release of weathered PAHs and similar to the current study, the number of rings on the molecule was indirectly related to contaminant desorption. White and Kottler (2002) showed that at concentrations as low as 5.0 mM, the aqueous concentration of Al and Fe in soil slurries increased by 39 and 16 times, respectively, and the desorption of *p*,*p*′-DDE was subsequently increased. White et al. (2003b) made similar observations and observed that LMWOA amendments to soil could increase *p*,*p*′-DDE uptake into *C. pepo* ssp. *pepo* by up to 66%. A fundamental difference in methodology between the previously mentioned studies and that of the current work needs to be addressed. Yang et al. (2001), White and Kottler (2002), White et al. (2003b) and Subramaniam et al. (2004) all have the Tenax™ beads, which is serving as the sorbing medium for contaminants as the molecules desorb from the soil, in direct contact with the soil slurry. The Tenax™, being a high surface area carbon material, has a sorption potential significantly greater than that of the soil and effectively induces the release of the compounds from soil. Thus, Yang et al. (2001) and Subramaniam et al. (2004) report percentages of PAH desorption of 10–90% as the Tenax™ artificially drives the sorption/desorption

equilibrium toward contaminant release. We maintain that such techniques actually mask the likely more subtle effects that the organic acids or other chelating agents have on the soil structure and on contaminant fate. In the current study, where the C18 disk is the sorbing medium but is only in contact with the supernatant of the soil slurry after exposure to the organic acids, the maximum desorption rate is 10.1%, and we feel this technique is more appropriate for assessing the effects of chelating agents on contaminant fate in soil. Lastly, the findings of differential desorption patterns among the 3–4 and 5–6 ring PAH consitutents with increasing LMWOA concentration is noteworthy. The mechanism of contaminant release is a function of soil matrix destruction via the organic acids, and thus, should not be directly influenced by physicochemical properties of the contaminants. These findings suggests that the 5–6 ring PAHs may actually be sorbed or sequestered in sites that are structurally different than the sorption sites of the lower molecular weight constituents, and that the sites retaining the more hydrophobic and recalcitrant molecules are more susceptible to the effects of chelating agents.

In the current study, the reasons for the failure to observe increased PAH desorption with hydroponically isolated solutions as a function of nutrient status are not known but are likely due to dilute concentrations of the organic acids as a function of the collection procedure. In a separate study, Gent et al. (2005) reported citric and malic acid exudation rates in a similarly designed experiment that ranged from 2.0 to 7.6 nmol/day/g plant material (dry weight). Given a 24 h collection period and estimated plant biomass of 2–3 g dry weight (typical of experiments in the current study), it is evident that the concentration of LMWOA was several orders of magnitude below that of the previous studies involving synthetic organic acids. Values in the literature for rates of organic acid exudation vary widely but recent work from our laboratory indicates that in the rhizosphere pore water of zucchini grown in soil-based rhizotrons, organic acid content may be at least an order of magnitude greater than that observed under dilute hydroponic conditions (Eitzer et al., submitted for publication). For hydroponic-derived exudates to be useful, the solutions will likely have to be concentrated to maximize visualization of differences in exudation patterns among plant species and as a function of nutrition. Such studies are currently being designed.

4. Conclusions

The findings that *C. pepo* ssp. *pepo* accumulates significantly greater quantities of highly weathered PAHs, including up to three orders of magnitude greater levels of the six ring constituents, further demonstrates the unique potential of this plant system. However, the 85% decrease in contaminant accumulation in ssp. *pepo* over consecutive growing seasons indicates that bioavailability of these hydrophobic residues in soils is still the rate-limiting factor for remediation. The accumulation of PAHs by two worm species, although significantly different and in general agreement with other reports in the literature, was at least an order of magnitude below levels found in the roots of various plant species. These data, taken with the highly variable accumulation of different POPs by worm and plant species reported in the literature, highlight the importance of receptor choice in efforts to estimate contaminant exposure, as well as remediation potential.

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